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Dysbiosis and Associated Stool Features Improve Prediction of Response to Biological Therapy in Inflammatory Bowel Disease

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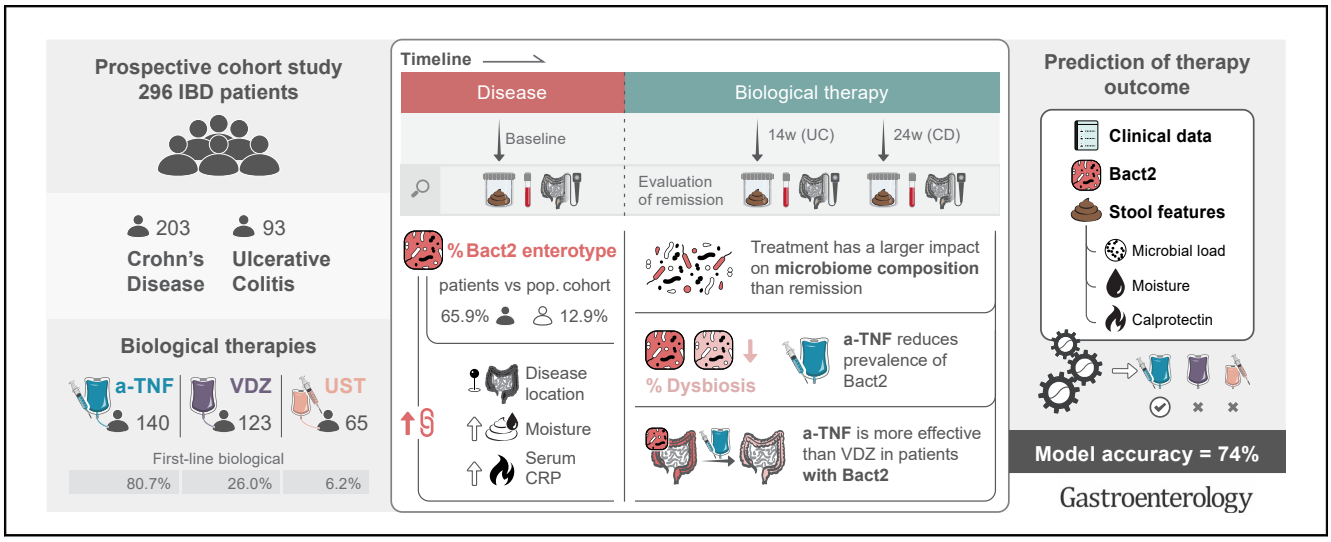

BACKGROUND & AIMS: Dysbiosis of the gut microbiota is considered a key contributor to inflammatory bowel disease (IBD) etiology. Here, we investigated potential associations between microbiota composition and the outcomes to biological therapies. **METHODS:** The study prospectively recruited 296 patients with active IBD (203 with Crohn's disease, 93 with ulcerative colitis) initiating biological therapy. Quantitative microbiome profiles of pretreatment and posttreatment fecal samples were obtained combining flow cytometry with 16S amplicon sequencing. Therapeutic response was assessed by endoscopy, patient-reported outcomes, and changes in fecal calprotectin. The effect of therapy on microbiome variation was evaluated using constrained ordination methods. Prediction of therapy outcome was performed using logistic regression with 5-fold cross-validation. **RESULTS:** At baseline, 65.9% of patients carried the dysbiotic *Bacteroides2* (Bact2) enterotype, with a significantly higher prevalence among patients with ileal involvement (76.8%). Microbiome variation was associated with the choice of biological therapy rather than with therapeutic outcome. Only anti–tumor necrosis factor-α treatment resulted in a microbiome shift away from Bact2, concomitant with an increase in microbial load and butyrogen abundances and a decrease in potentially opportunistic *Veillonella.* Remission rates for patients hosting Bact2 at baseline were significantly higher with anti–tumor necrosis factor-α than with vedolizumab (65.1% vs 35.2%). A prediction model, based on anthropometrics and clinical data, stool features (microbial load, moisture, and calprotectin), and Bact2 detection predicted treatment outcome with 73.9% accuracy for specific biological therapies. **CONCLUSION:** Fecal characterization based on microbial load, moisture content, calprotectin concentration, and enterotyping may aid in the therapeutic choice of biological therapy in IBD.

Keywords: Inflammatory Bowel Disease; Biological Therapy; Anti-TNF; Anti-integrin; Anti-IL12/23; Quantitative Microbiome Profiling; Fecal Calprotectin; Enterotype; Inflammation; Therapy Outcome Prediction.

Inflammatory bowel diseases (IBDs) comprise a range of complex multifactorial disorders of the gastrointestinal tract triggered by both innate and adaptive immune responses to environmental factors in often genetically predisposed individuals.[1,2] A crucial role in disease etiology and activity appears, however, to be played by the patient's gut microbiota.[3] The microbial composition of fecal samples of individuals with IBD has been shown to diverge fundamentally from the communities observed in samples obtained from healthy individuals.[4–6] Generally referred to as dysbiotic, the imbalanced IBD microbiota has been shown to be characterized by low microbial diversity,[7] low bacterial cell counts,[6] and reduced proportional as well as absolute abundances of commensal microorganisms,[8] including colonic butyrate producers previously identified as essential for intestinal homeostasis.[9]

During remission after anti-inflammatory therapy, a diversification of the microbiota toward a more eubiosis-like composition has been observed.[4,6,10–17] Moreover, respectively high and low baseline proportion of short-chain fatty acid–producing and mucus-degrading bacteria have been associated with a higher probability of a favorable outcome of therapeutic interventions.[13,18–20] Additionally, the absence or reduced proportions of taxa exhibiting proinflammatory properties, such as *Fusobacterium*, *Veillonella*, *Escherichia*, and *Streptococcus*, has been described to increase the likelihood of achieving remission after medical treatment.[6,12,14,20,21]

The IBD therapeutic armamentarium has expanded substantially during the last decade; however, reliable tools allowing the prediction of therapy outcome, patient-specific treatment success, or probability of and time to relapse are still lacking.[22] Although their clinical value is beyond doubt, the development of such tools and the underlying statistical models require a substantial investment in cohort assembly, comprising characterization of baseline activity as well endoscopic evaluation of treatment outcome.

Two additional matters complicating the design of robust models are the heterogeneity of IBD presentations beyond the classic Crohn's disease (CD) and ulcerative colitis (UC) diagnoses and the range of biological pathways targeted by currently available therapies, often with varying efficacy depending on disease (sub)phenotype.[23,24] As a result, although some of the currently available models have proposed to predict treatment outcome with reasonable accuracy, they tend to focus on a single diagnosis, assessing the probability of attenuation of inflammation for a specific treatment.[18,19,25]

To address these challenges and to evaluate potential associations between patients' fecal microbiota composition and biological therapy outcome, the present prospective study monitored changes in bacterial communities associated with a therapeutic intervention with 3 distinct biological therapies in a large IBD cohort. Linking disease subtypes, clinical data, and biological therapy with stool characteristics, microbiome data, and treatment outcome, we develop and validate a predictive model that could assist clinicians in determining a patient-specific therapeutic strategy.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Dysbiosis of the gut microbiota contributes to inflammatory bowel disease pathogenesis and could affect response rates of biologicals.

NEW FINDINGS

Anti-tumor necrosis factor-α had the largest impact on microbiota composition, and vedolizumab appeared less effective among patients harboring a dysbiotic microbiota.

LIMITATIONS

Given the prospective study design, vedolizumab was mostly administered as a second- or third-line treatment.

CLINICAL RESEARCH RELEVANCE

A model based on anthropometrics/clinical data, stool features, and detection of dysbiosis allowed the prediction of treatment outcome of different biologicals with 73.9% accuracy.

BASIC RESEARCH RELEVANCE

Longitudinal studies capitalizing on the microbiota-modulating effect of anti–tumour necrosis factor-α should allow establishing a chain of events and determining whether restoration of eubiosis precedes, follows, or coincides with endoscopic remission.

Materials and Methods

Ethical Compliance

All experimental protocols were approved by the Universitair Ziekenhuis (UZ) Leuven Ethische Commissie Onderzoek, (UZ Katholieke Universiteit [KU] Leuven reference number: S53684). The study design complied with all relevant ethical regulations, aligning with the Declaration of Helsinki and in accordance with Belgian privacy laws. All participants provided signed informed consent.

Sample Collection

A total of 296 IBD patients with active disease (203 CD and 93 UC) initiating biological therapy (140 with anti–tumor

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Abbreviations used in this paper: **Adj, adjusted; a-TNF, anti–tumor necrosis factor-α; Bact2, *Bacteroides2* enterotype; BMI, body mass index; CRP, C-reactive protein; CD, Crohn's disease; dbRDA, distance-based redundancy analysis; IBD, inflammatory bowel disease; L1, ileal; L2, colonic; L3, ileocolonic; Np/np, total number/subset of patients; Ns/ns, total number/subset of samples; RR, relative risk; SES-CD, simple endoscopic score for Crohn's disease; UC, ulcerative colitis; UST, ustekinumab; VDZ, vedolizumab.**

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necrosis factor-α [a-TNF], 123 with vedolizumab [VDZ], and 65 with ustekinumab [UST]) were recruited when attending the IBD outpatient clinic of the University Hospitals Leuven. Diagnosis of IBD was established on clinical symptoms, endoscopy, imaging, and histology following the European Crohn and Colitis Organisation guidelines.[26] Detailed inclusion and exclusion criteria are provided in the Supplementary Methods.

Fecal samples were collected at baseline and week 14 for UC and at baseline and week 24 for patients with CD, aligned to endoscopic outcome evaluation. Baseline stool samples were collected a maximum of 4 weeks before endoscopy and always before bowel preparation. Serum concentrations of C-reactive protein (CRP), albumin, and hemoglobin, and fecal levels of calprotectin were determined at baseline and before outcome evaluation.

Therapy Outcome Evaluation

Treatment outcome was determined through endoscopic evaluation at week 14 for patients with UC, in accordance with the policy of the Belgium public health insurance. For patients with CD, endoscopic evaluation was performed according to standard practice after 24 weeks. For UC, endoscopic remission was defined as Mayo endoscopic subscore $\leq$1, with at least a 1-point decrease from baseline. For CD, endoscopic remission was defined as simple endoscopic score for CD (SES-CD) $\leq$3 (SES-CD 0 for patients starting with SES-CD 3) or absence of ulceration, or both.[27]

Clinical Profiling

Patient age, disease duration (months), biological history (number of prior biological therapies), disease location and extent (Montreal classification), concomitant medication, previous surgery, body mass index (BMI; kg/m^2), smoking (active smoking or past/nonsmoker), and sex (male/female) were extracted from their clinical records.

Fecal Sample Collection

Fecal samples were collected at home and stored in the freezer (-20°C) for a maximum of 24 hours until transport to the research facility in a cooling bag. At the research facility, samples were stored at -80°C.

Fecal Sample Characterization and Microbiota Profiling

Fecal samples were analyzed for calprotectin levels, moisture, and microbial load determinations. The composition of the fecal microbiota was profiled by quantitative amplicon sequencing, as described in the Supplementary Methods.

Statistical Analysis

Statistics were performed in R software (The R Foundation for Statistical Computing) using the community ecology package *vegan*[28] and the microbiome-specific package *phyloseq*.[29] All statistical tests were 2-sided. *P* values were corrected for multiple testing using the Benjamini-Hochberg method (reported adjusted [Adj]*P*) when applicable. Prediction analyses were performed using Python package Scikit-learn.[30] See Supplementary Methods for more details on the statistical methods.

Results

Prospective, Multitherapy Cohort Enabled Microbiome Monitoring in Inflammatory Bowel Disease Treatment

To prospectively monitor associations between IBD treatment and microbiota composition, we recruited a cohort of 296 patients (203 with CD, 93 with UC) with active IBD (as established based on endoscopic evaluation) initiating biological therapy. During the study period, 328 biological therapies (interventions) were started up among cohort participants, including 140 a-TNF (infliximab, adalimumab, and golimumab), 123 anti-integrin (VDZ), and 65 anti-interleukin 12/23 (UST) treatments. Within the study time frame, 32 patients (17 CD, 15 UC) switched from one biological therapy to an alternative treatment. Consecutive treatments were treated as independent interventions (with updated biological history), but patient identifiers were conserved throughout analyses.

Given the prospective study design, the choice of therapy was not randomized but prescribed based on a combination of clinical guidelines, subphenotypes, availability of drugs, or patient/physician preference, or a combination of these. As a result, our analyses concern a-TNF mostly as a first-line therapy, whereas VDZ and UST were administered mainly as second-line therapy after lack or loss of response to prior treatment(s).

Baseline patient characteristics (anthropometrics and clinical data) were recorded at the start of each of the 328 interventions analyzed (Table 1). Participants were requested to collect a fecal sample at baseline (pretreatment) and before a preplanned visit for endoscopic outcome evaluation (week 14 for UC and 24 for CD). A total of 515 samples were collected. A core cohort of 177 participants (120 CD, 57 UC) initiating 187 interventions (77 a-TNF, 63 VDZ, and 47 UST, with 2 CD and 8 UC patients receiving 2 consecutive therapies) (Supplementary Table 1) effectively provided a fecal sample both at baseline and at outcome evaluation.

Fecal samples were analyzed with quantitative microbiome profiling,[31] combining 16S ribosomal RNA gene amplicon sequencing with flow cytometry, to evaluate potential associations between genus-level microbiota composition and therapy outcome. An overview of the impact of the biological therapies on patients' clinical readouts and fecal characteristics is presented in Supplementary Table 2.

Microbiome Composition in Patients With Active Inflammatory Bowel Disease Is Linked to Disease Location

First, we screened for covariates of quantitative fecal microbiome diversification among baseline samples. Potential covariates covered participant characteristics (age, sex, BMI, and smoking status), blood panel results (CRP, albumin, and hemoglobin), disease descriptors (diagnosis [UC/CD], disease location [Montreal classification], and disease duration), current medication (steroids, antibiotics,

Table 1. Patient Demographics and Baseline Characteristics

	Full cohort (N = 328)			CD (n = 220)			UC (n = 108)	
	a-TNF	VDZ	UST	a-TNF	VDZ	UST	a-TNF	VDZ
Variable	(n = 140)	(n = 123)	(n = 65)	(n = 83)	(n = 72)	(n = 65)	(n = 57)	(n = 51)
Female sex	57.1	60.2	69.2	53	62.5	69.2	63.2	56.9
Age, *y*	38.9 (15.9)	45.3 (16.2)	42.2 (14.5)	38.5 (15.8)	43.5 (17.2)	42.4 (14.5)	39.5 (16.0)	47.9 (14.6)
Fecal calprotectin, *μg/g*	1249.2 (777.2)	940.2 (723.8)	1062.6 (756.1)	1099.1 (738.6)	844.7 (707.8)	1062.6 (756.1)	1477.1 (785.9)	1076.8 (731.8)
CRP, *mg/L*	5.7 (1.9–21.9)	3.6 (1.3–10.3)	9.9 (3–16.4)	6.5 (2.2–24.7)	4.2 (1.7–14.2)	9.9 (3–16.4)	4.7 (1.4–15.3)	2.7 (0.9–7.0)
Disease duration, *y*	7.9 (9.8)	12.2 (10.8)	16.1 (10.3)	8.7 (11.1)	13.9 (11.4)	16.1 (10.3)	6.7 (7.3)	9.9 (9.6)
BMI, kg/m^2	23.5 (4.2)	25.2 (4.4)	23.9 (5)	23.3 (4.2)	24.8 (4.4)	23.9 (5)	23.8 (4.1)	25.7 (4.4)
Active smoking	10.7	10.6	21.5	16.9	16.7	21.5	1.8	2
SES–CD score				9 (5–15)	12 (8–17)	8 (5–12)		
Mayo endoscopic score[a]								
1							3.5	7.8
2							45.6	45.1
3							47.4	47.1
Disease location								
L1				39.8	40.3	41.5		
L2				20.5	16.7	24.6		
L3				37.3	38.9	33.8		
Disease extent								
Proctitis E1							17.5	19.6
Left–sided colitis E2							56.1	52.9
Extensive colitis E3							26.3	25.5
Concomitant mesalamine	34.3	30.9	3.1	9.6	2.8	3.1	70.2	70.6
Concomitant antibiotics	8.6	4.1	7.7	12	5.6	7.7	3.5	2
Concomitant PPIs	19.3	25.2	26.2	20.5	30.6	26.2	17.5	17.6
Concomitant steroids	43.6	37.4	33.8	38.6	38.9	33.8	50.9	35.3
Concomitant azathioprine	17.1	12.2	0	16.9	13.9	0	17.5	9.8
Previous biologicals								
0	80.7	26	6.2	80.7	22.2	6.2	78.9	31.4
1	14.3	35.8	1.5	15.7	30.6	1.5	12.3	41.2
2	5	36.6	41.5	3.6	45.8	41.5	8.8	25.5
3	0	1.6	35.4	0	1.4	35.4	0	2

Table 1. Continued

Variable	Full cohort (N = 328)			CD (n = 220)			UC (n = 108)	
	a-TNF (n = 140)	VDZ (n = 123)	UST (n = 65)	a-TNF (n = 83)	VDZ (n = 72)	UST (n = 65)	a-TNF (n = 57)	VDZ (n = 51)
Previous surgery				28.9	40.3	49.2		
Responders	61	44.8	10.8	72,0	36.9	10.8	52.6	54.9

NOTE. Data are presented as percentage (%), mean (standard deviation), or median (interquartile range).
PPI, proton pump inhibitor.
[a]Two patients in the a-TNF group had Mayo endoscopic score of 0.

immunosuppressants, and proton pump inhibitors), treatment history (number/type of prior biologicals and prior immunosuppressants), fecal characteristics (stool moisture and calprotectin), and allocation to therapy. History of surgical interventions and current mesalamine intake were excluded from the screening due to their close association with disease location (likelihood ratio test, R^2 = 0.22, Adj$P$ = 1.01e−16, number of patients [np] = 291) and diagnosis (R^2 = 0.41, Adj$P$ = 4.88e−36, Np = 296), respectively (Supplementary Figure 1 and Supplementary Table 3). Disease location, fecal moisture content, and patients' inflammatory status (serum CRP concentration) were identified as presenting both significant and nonredundant associations with genus-level microbial community variation (stepwise distance-based redundancy analysis [dbRDA], full model R^2 = 2.08%, Adj$P$ = 2e−04, np = 281/number of samples [ns] = 311) (Figure 1*A* and Supplementary Table 4). Fecal moisture and systemic inflammation levels were not significantly intercorrelated (Spearman correlation, P = .68, np/ns = 291/323) (Supplementary Table 3).

Therapy allocation did not emerge as a significant, nonredundant covariate of baseline microbiome community composition (stepwise dbRDA, Adj$P$ = .086, np/ns = 281/311) (Supplementary Table 4), confirming a reasonably balanced distribution of patients over the intervention groups in baseline microbiota composition. However, it should be noted that when singled-out (single dbRDA, R^2 = 1.13%,Adj$P$ = .025, Np/ns = 296/328), the variable did display an association because UST was prescribed only to patients with CD (Supplementary Figure 1). The composition of baseline fecal samples did not covary with calprotectin as a marker of local inflammation (single dbRDA, P = .070; np/ns = 285/315) or with previous exposure to biologicals (P = .412, Np/ns = 296/328).

Disease location, a variable stratifying patients according to the Montreal classification,[32] distinguishing between ileal (L1CD; np/ns = 85/89), colonic (L2CD/UC; np/ns = 133/153), and ileocolonic (L3CD; np/ns = 78/81) presentations (grouping colonic L2CD patients with UC patients; excluding patients with uncharacterized location [np/ns = 5/5]), was more closely associated with baseline microbiota variation than the binary discrimination between CD and UC diagnoses (single dbRDA, disease location, R^2 = 1.60%, Adj$P$ = .017, np/ns = 291/323; diagnosis, R^2 = 1.03%, Adj$P$ = .017, Np/ns = 296/328) (Supplementary Table 4). The Montreal classification was observed to be linked to patient inflammatory status (CRP, Kruskal-Wallis Adj$P$ = .003, np/ns = 290/322; calprotectin, Adj$P$ = 1.6e−12, np/ns = 280/310), with the lowest systemic (CRP) and gastrointestinal (calprotectin) inflammation levels found in ileal (L1CD presentations (Supplementary Figure 2).

Analyzing individual taxa abundances in baseline samples, we identified 12 bacterial genera as diverging in numbers between disease locations (Kruskal-Wallis Adj$P$ < 0.05, np/ns = 291/323) (Supplementary Table 5). Ten of those were increased in colonic IBD subtypes, whereas only *Fusobacterium* (Kruskal-Wallis, Adj$P$ = 2.03e−3, np/ns = 291/323) and *Bilophila* (Adj$P$= 3.87e−3, np/ns = 291/323)

abundances were higher in the exclusively ileal presentation (Supplementary Table 5). A correlation analysis of the abundances of these 12 genera (excluding samples in which both taxa were absent) showed that all significant associations with *Fusobacterium* (11 associations) or *Bilophila* (5 associations) were coexclusions (Spearman's correlation Adj$P < .05$) (Supplementary Table 6).

An alternative approach in analysing microbiome compositional variation consists in reducing the complexity of the gut microbiota by classification into community types, also referred to as enterotypes.[33] The recent adoption of appropriate community-typing methods (Dirichlet multinomial mixtures[34] has consistently stratified Western human gut microbiome variation across studies into 4 enterotypes.[31,35,36] Among these, the configuration labelled as *Bacteriodes*2 (Bact2) has been shown to group fecal samples with the key characteristics of a perturbed (dysbiotic) microbiota, including an overall decrease in microbial load (microbial cells per gram of feces), a depletion of butyrate-producing bacteria, and an association with systemic (CRP) as well as gastrointestinal (fecal calprotectin) inflammation.[6,31,36] Applying a Dirichlet multinomial mixtures approach (grouping samples potentially originating from a same community based on probabilistic models without making any claims regarding the putative discrete nature of the strata detected), we here confirmed the presence of 4 enterotypes in our prospective cohort (Figure 1*B*). Overall, 63.7% of the 515 fecal samples were classified as Bact2 (65.9% in baseline samples; ns = 328). Keeping 1 random sample per patient (single intervention subset, Np = 296), again 65.9% of patients harbored a Bact2 microbiota at baseline, largely exceeding the 12.9% prevalence previously observed in a 1120 cross-sectional population cohort studied in the same geographic region as the present sampling effort (Flemish Gut Flora Project[37]; 2-tail Fisher's exact test, $\chi^2 = 583$, Adj$P = 8.46e{-}129$). These findings confirm our previous observation of a high prevalence of the aberrant Bact2 community type among patients with IBD in a cross-sectional study,[6] an observation that we linked to accelerated transit as well as higher intestinal inflammation levels.

By modelling the association between Bact2 detection in baseline samples and the 3 significant drivers of microbiota variation identified above (disease location, fecal moisture, and serum CRP concentrations; multivariate logistic regression, $R^2 = 8.15\%$, $P = .007$, np/ns = 286/318) (Figure 1*C–E* and Supplementary Table 7), we observed both loose stools (fecal moisture content relative risk [RR] = 1.54, Adj$P = 7e{-}4$) and systemic inflammation (CRP [$\log_{10}$] RR = 1.31, Adj$P = .012$) (Figure 1*C* and *D*) were linked with an increased risk of hosting a Bact2 community.

Regarding disease location, we found the dysbiotic Bact2 community type occurred less frequently among patients with a colonic disease presentation (L2CD/UC, RR = 0.55, Adj$P$ = 7.0e-4; L3CD, RR = 0.72, Adj$P = .040$) (Supplementary Table 7). Indeed, baseline detection of Bact2 among patients with an ileal (L1CD) presentation was higher compared with L2CD/UC patients (L1CD, 76.8% vs L2CD/UC, 57.1%; $\chi^2 = 9.24$, Adj$P = .005$, Np = 296 [single-intervention subset]) (Figure 1*E*), whereas an intermediate prevalence was noted among individuals with an ileocolonic manifestation (L3CD, 68.4%) (Supplementary Table 8).

Anti-Tumor Necrosis Factor-α Therapy Modulates Patient Microbiome Composition and Enterotype Prevalence

As a second set of analyses, we assessed the impact of treatment and treatment response on microbiome variation observed among preintervention and postintervention samples. Using endoscopic evaluation as a gold standard to define treatment response, we classified outcome evaluations as remission/nonremission (281 categorized in 253 patients of 328 interventions evaluated), observing an overall remission rate of 42.70%. Significantly different remission rates were obtained with different biologicals (contingency test, $\chi^2 = 45.81$, $P = 1.54e{-}10$, n = 281). A higher endoscopic remission rate was achieved with a-TNF (61.00%, n = 100) compared with VDZ (44.83%, n = 116) and UST (10.77%, n = 65) (Figure 2*A* and Supplementary Table 9).

When stratified by disease location (omitting UST interventions given their unbalanced administration with respect to disease location), significantly higher remission rates for a-TNF compared with VDZ were confirmed in patients with ileocolonic presentations (Adj$P = .03$, n = 36), but not among ileal CD (Adj$P = .13$, n = 48) or patients with colonic disease (Adj$P = .47$, n = 128) (Supplementary Figure 3 and Supplementary Table 9).

When stratified by diagnosis, remission rates between a-TNF- and VDZ-treated patients were only significantly different among CD patients (72.09%, n = 43 vs 36.92%, n = 65, $\chi^2 = 13.15$, Adj$P = 6.00e{-}4$). However, it should be noted that when we restricted our analyses to first-line interventions (omitting first-line UST [n = 4]), no significant differences in endoscopic remission rates between a-TNF (65.00%, n = 80) and VDZ (60.00%, n = 30) could be observed, regardless of location- or diagnosis-based stratification (Supplementary Table 9).

Interindividual differences have been shown to explain most of the human gut microbiota compositional variance.[37] Also in the present prospective cohort (preintervention as well as postintervention samples), quantitative fecal microbiome composition was firmly associated with between-patient variation (stepwise dbRDA, patient identifier, $R^2 = 60.46\%$, Adj$P = .001$, np/ns = 273/446) (Figure 1*B*). Beyond individual differences, only therapeutic intervention—a 4-class variable categorizing samples as preintervention/postintervention, with the latter category being split according to the therapy allocated (a-TNF, VDZ, and UST)—provided a significant nonredundant contribution to genus-level microbial community variation ($R^2 = 1.79\%$, Adj$P = .0082$, np/ns = 273/446) (Figure 1*B*).

Disease location (stepwise dbRDA, Adj$P = .11$, np/ns = 273/446) and treatment response (coded as a 2-class variable [active/remission], with pretreatment samples and nonremission posttreatment samples categorized as active;

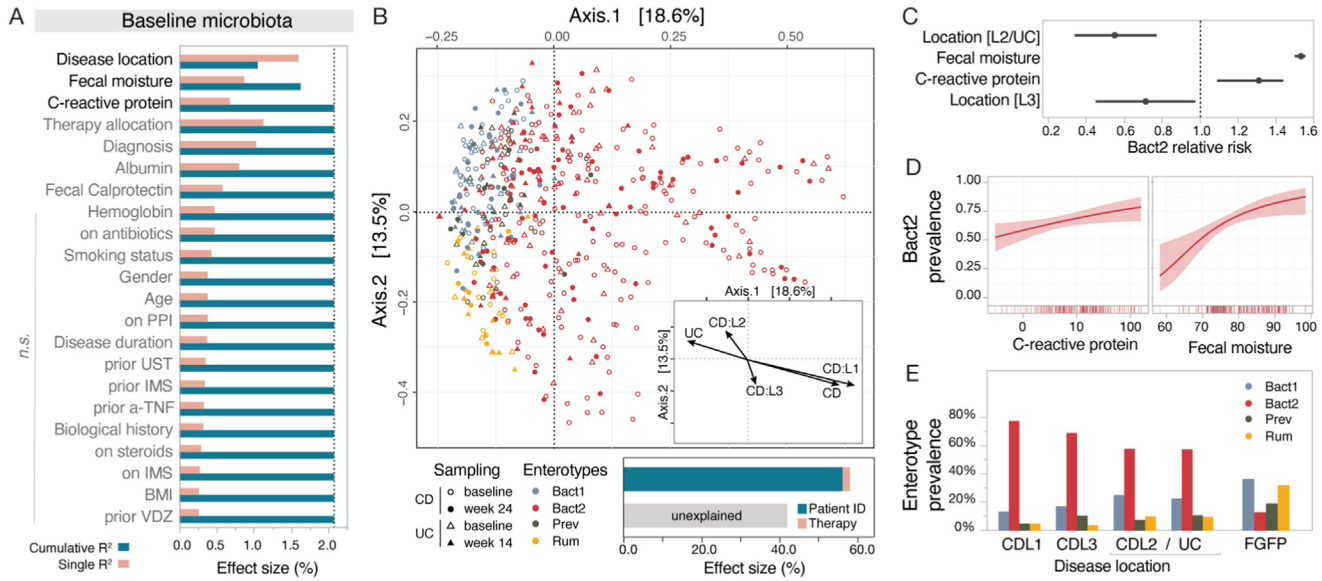

Figure 1. Gut microbiota composition in a cohort with active IBD during biological therapy (study cohort, Np = 296, Ns = 515). (*A*) Covariates of microbiota variation in baseline samples of the study cohort (ns = 328). Representation of explanatory power of patient's clinical variables and fecal characteristics used in a single variable model (single R^2) or combined in a stepwise model (cumulative R^2). The variables of disease location (L1CD, L2CD/UC, L3CD), fecal moisture content, and serum CRP contributing significantly to the stepwise model are listed in black (dashed line representing the total cumulative R^2), whereas gray labels have nonsignificant (n.s.) contributions. IMS, immunosuppressants. PPI, proton pump inhibitor. (*B*) Principal coordinates analysis visualization of interindividual differences in quantitative microbiome profiles within the IBD study cohort (Np = 296, Ns = 515). CD and UC diagnosis at baseline and at the endoscopic evaluation (week 14 for UC and 24 for CD) are represented by shape, whereas colors represent enterotypes. *Insert*: Post hoc fit of the microbiota variation associated to ileal (L1CD), colonic (L2CD/UC), and ileocolonic (L3CD) presentations. Bar plot representation of gut microbiota variation partitioning in the study cohort. The significant covariates of interindividual variation (patient ID) and intervention (baseline, a-TNF, VDZ, and UST after intervention) cumulatively explain 58% of microbiome variation (stepwise dbRDA). ID, identifier. (*C*, *D*, and *E*) Modeling the association between the prevalence of the dysbiotic Bact2-enterotype and the 3 significant drivers of microbiota variation (logistic regression, ns = 318). (*C*) Relative risk of patients being Bact2-enterotype carriers associated with the significant drivers of microbiota variation described in panel *A*. (*D*) Logistic regression visualization of the increased prevalence of Bact2-enterotype carriers with increasing serum CRP levels and fecal moisture. (*E*) Prevalence of Bact2-enterotype carriers in the study cohort (single-intervention subset, ns = 296 baseline samples) stratified according to disease location and compared with the Flemish Gut Flora Project (FGFP) population study data set (N = 1120, contingency tests).

Adj$P$ = .84, np/ns = 273/446) both failed to contribute significantly to microbial community variation beyond intervention and interindividual variation in the present prospective cohort (Supplementary Table 10). Hence, our analyses identified choice of biological therapy to be more strongly associated with microbiota composition variation than across-treatment patient response.

To evaluate the specific impact of the interventions studied on quantitative microbiome composition, we focused on a core cohort only comprising patients who provided fecal samples before therapy initiation as well as at outcome evaluation (np = 177, ns = 187 pairs of samples, including 1 individual with undefined disease location) (Supplementary Figure 4). Paired statistics were applied to assess changes in microbiome composition between baseline and study end point. A significant change in gut microbiota composition was associated with a-TNF therapy (paired dbRDA, R^2 = 2.15%, Adj$P$ = .0017, np = 77) but not with VDZ intervention (Adj$P$ = .35, np = 63) or treatment with UST (Adj$P$ = .48, np = 47) (Figure 2*B* and Supplementary Table 11). Also when the core data set was restricted to interventions leading to endoscopic remission, a significant impact on microbiome variation could only be established for a-TNF (R^2 = 3.48%, Adj$P$ = .04, np = 32) (Figure 2*B*), confirming that the former observation was not driven by the higher a-TNF remission rate. Stratification by diagnosis (full core cohort) confirmed that changes in microbiome composition after a-TNF (and not VDZ or UST) therapy occurred both in UC (R^2 = 4.05%, Adj$P$ = .04, np = 29) and CD patients (R^2 = 2.16%, Adj$P$ = .04, np = 48) (Supplementary Table 11).

Microbiota change associated with a-TNF therapy additionally translated into a minor enterotype switch (logistic regression on Bact2 carrier status marginalizing patient identifier, χ^2 = 4.08, P = .044, np = 77), with 6 of 47 carriers (12.8%) shifting away from the potentially dysbiotic Bact2 enterotype (Figure 2*C*). We identified 20 taxa with significant changes in quantitative abundances after a-TNF treatment (Wilcoxon's signed rank test, np = 77) (Supplementary Table 12 and Figure 2*D*), 19 of which increased during intervention. These included key gut-associated butyrogens such as *Roseburia*, *Blautia*, *Subdoligranulum*, *Anaerostipes*, *Ruminococcus*, and *Butyricicoccus*. In contrast, *Veillonella*, a genus previously put

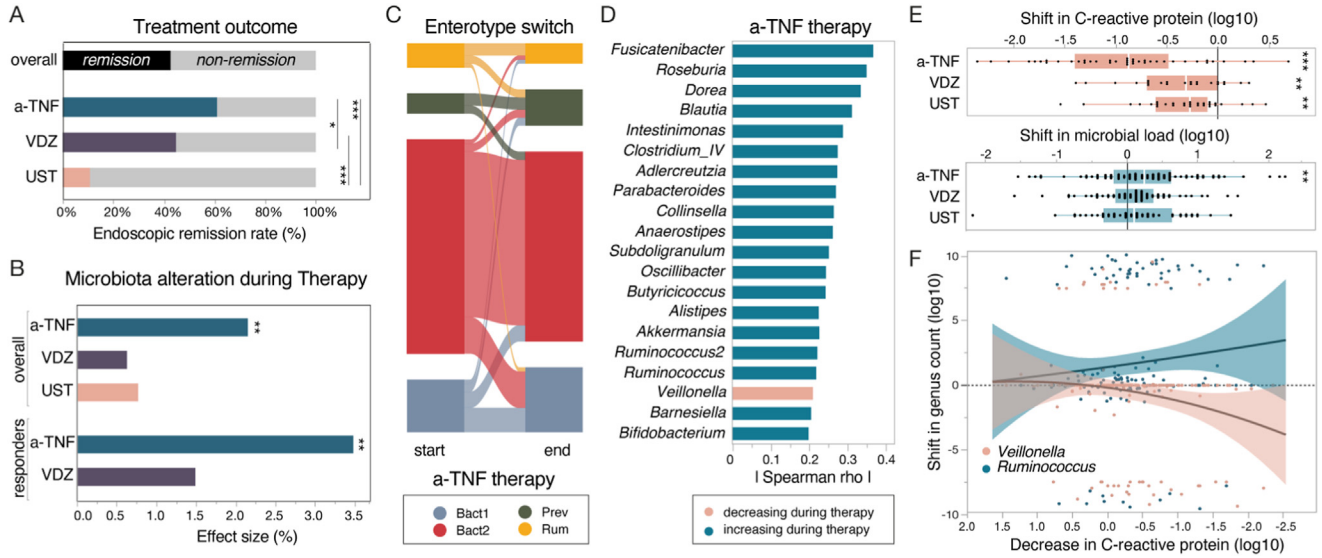

Figure 2. Effect of biological therapy intervention on gut microbiota composition in patients with active IBD (core cohort, np = 187, ns = 187 × 2). (*A*) Percentage of interventions resulting in endoscopic remission for treatment with a-TNF (np = 100), VDZ (np = 116), and UST (np = 65). (*B*) Microbiota variation correlated to biological therapies with a-TNF, VDZ, and UST (paired dbRDA R^2) for 187 patients and restricting to 66 patients having achieved endoscopic remission. (*C*) Alluvial diagram represents patients switching in enterotypes from baseline to postintervention in the core cohort for a-TNF therapy (np = 77). Rum, *Ruminococcus*. (*D*) Genera significantly associated with a-TNF intervention are shown. Bars represent the absolute effect size of the difference in quantitative genera abundances between baseline and the end of the intervention (np = 77, Wilcoxon's test), with positive and negative associations in blue and pink, respectively. Only significant associations are listed (Wilcoxon's test Adj$P$ < .05). (*E*) Shift in serum CRP (for patients with baseline CRP levels above the clinical threshold of >5 mg/L) and fecal microbial load during biological therapy intervention with a-TNF, VDZ, or UST. Shifts are defined as the difference in log-value between samples collected preintervention and postintervention. Significant differences over the intervention are indicated (np = 187) by Wilcoxon's signed rank test. *Adj$P$ < .05, **Adj$P$ < .01, ***Adj$P$ <0.001. (*F*) Genera with abundance shift significantly associated to the shift in serum CRP during a-TNF intervention in the core cohort (np = 77). Shifts from preintervention to postintervention were defined as in panel *E*. Only *Veillonella* and *Ruminococcus* abundances were significantly correlated with the shift in CRP, respectively decreasing and increasing with the decrease in CRP associated with a-TNF intervention.

forward as a potential opportunist in inflammatory conditions,[6] was the only taxon significantly decreasing in abundance after a-TNF therapy (Wilcoxon's signed rank test, effect size = −0.21, Adj$P$ = .0290, np = 77). No significant changes in taxa abundances were observed to be associated with VDZ or UST interventions (Supplementary Table 12). Although these observations seem to make a case of a-TNF being the strongest microbiota-modulating agent among the therapies evaluated, it remains important to emphasize that in the present prospective study, VDZ and UST were mostly administered as second-line treatments.

Given the identified associations between baseline microbiota composition, fecal moisture, and systemic inflammation, we assessed whether these microbiome covariates were impacted by treatment in the core cohort (disease location remaining unchanged). Stool moisture was not altered significantly by any of the biologicals studied (Wilcoxon's signed rank test, a-TNF, Adj$P$ = .34, np = 76; VDZ, Adj$P$ = .34,np = 63; UST, Adj$P$ = .55, np = 47) (Supplementary Table 13). All patients had active disease at baseline (endoscopy), but this was not always reflected by elevated serum CRP levels. When the analysis was restricted to patients with baseline CRP levels above the clinical threshold (>5 mg/L), all 3 therapies were observed to result in a significant lowering of serum CRP levels (Wilcoxon's signed rank test, a-TNF, Adj$P$ = 1.10e−6, np = 41; UST, Adj$P$ = 1.08e−3, np = 29; VDZ, Adj$P$ = 4.65e−3, np = 22) (Figure 2*E*), the largest effect being associated with a-TNF therapy (a-TNF, r = −0.58; UST, r = −0.48; VDZ, r = −0.48).

Moreover, only a-TNF treatment was additionally accompanied by a significant increase in fecal microbial load (r = 0.24, Adj$P$ = 6.41e−3, n = 77) (Figure 2*E* and Supplementary Table 13), in line with the observed changes in microbiome variation and suggesting the post–a-TNF gut environment is more favorable to gut microbial community reestablishment. However, post–a-TNF fecal microbial loads remained significantly lower than the levels observed in samples from healthy controls (np = 273 healthy controls cohort[37]; r = 0.48, Adj$P$ = 1.34e−18, np = 350) (Supplementary Figure 5). Of the 20 genera responsive to a-TNF therapy, *Veillonella* and *Ruminococcus* were the only taxa for which the magnitude of their abundance shift was correlated with the observed decrease in serum CRP levels (although not surviving multiple testing correction). Although the abundance of *Veillonella* decreased with decreasing inflammation levels (Pearson's correlation, r = 0.27, P = .019, np = 77), *Ruminococcus* followed the opposite trend (Pearson's correlation, r = −0.25, P = .028, np = 77) (Figure 2*F* and Supplementary Table 14).

Despite this association between *Veillonella* and *Ruminococcus* abundance shifts and dampening of inflammatory tone during treatment, none of the 20 taxa associated to the a-TNF intervention were significantly different when a-TNF responders were compared with nonresponders (Supplementary Table 14). We could not detect a significant correlation between the observed increase in fecal cell density and the decrease of systemic inflammation levels (Pearson's correlation, $P = .40$, np = 77).

Anti-Tumor Necrosis Factor-α Treatment Results in Higher Therapeutic Response Rates Among Patients Hosting a Dysbiotic Microbiota

Next, we evaluated whether occurrence of remission after biological therapy could be modelled by baseline clinical status or gut microbiota composition of patients. For each treatment, we assessed potential associations between intervention response (endoscopic evaluation of remission/nonremission) and patient baseline characteristics/clinical status (sex, age, BMI, inflammatory status [CRP and fecal calprotectin], smoking status, disease location, duration, and refractoriness [number of prior biological classes used before this intervention]) and microbiota (Bact2 carrier status, fecal moisture, and microbial load). Using these 12 variables to predict intervention response (logistic regression), we found only VDZ treatment outcome was associated with baseline characteristics (logistic regression, a-TNF, Adj$P = .33$, n = 87; VDZ, Adj$P = .016$, n = 107; UST, Adj$P = .17$, n = 65) (Supplementary Table 15). Importantly, in the present study neither disease location nor diagnosis (UC/CD) were predictive of endoscopic remission for either of the therapies evaluated (single-variable logistic regression $P > .05$) (Supplementary Table 15). According to the model, a positive Bact2 carrier status (estimate = −0.90, $P = .038$, n = 110) and higher fecal calprotectin levels (estimate = −0.83, Adj$P = .045$, n = 110) both decreased the chance of achieving remission after VDZ therapy almost by half (RR = 0.55 and RR = 0.59, respectively, and nonredundantly) (Figure 3*A*). As a result, remission rates for patients hosting Bact2 microbiota at baseline were markedly and significantly higher using a-TNF compared with VDZ (65.08% of n = 63 vs 35.21% of n = 71, respectively; Fisher's exact test, $\chi^2 = 12.10$, $P = .003$, n = 134) (Figure 3*B* and Supplementary Table 9), a trend confirmed when our analyses were restricted to first-line interventions (69.81% of n = 53 vs 52.63% of n = 19, respectively; $\chi^2 = 1.78$, $P = .14$, n = 72) (Supplementary Table 9).

Analyzing recovery from dysbiosis to eubiosis upon treatment with a-TNF and VDZ showed that the proportion of Bact2 carriers recovering a eubiotic enterotype when achieving remission was similar for both treatments (a-TNF, 40.00%; VDZ, 35.29%) and higher than observed among nonresponders (a-TNF, 15.38%; VDZ, 10.53%; all responders vs nonresponders, Fisher's exact test, $\chi^2 = 6.01$, $P = .027$, n = 69) (Supplementary Table 16). Switching from dysbiosis to a eubiotic enterotype was not correlated to any patient baseline characteristics and only associated to a positive endoscopic outcome (single logistic regression, estimate = 1.45, $P = .022$, n = 69) (Figure 3*C*).

Assessment of Bacteroides2 *and Associated Stool Features Enables Prediction of Therapy Response*

Over the years, multiple models have been proposed to predict therapy success in IBD based on a combination of patients' clinical data and microbiome profiles. To date, such models have, however, been restricted to the administration of a single biological therapy.[18–20] Here, capitalizing on the sample size and richness of the data collected, we created a model that would allow the identification of a preferential therapy with highest probability of achieving remission of the biologicals studied. To do so, we categorized the available metadata into 3 sets of variables according to (decreasing) ease of sample collection and data acquisition; namely, (1) patient clinical data (age, sex, BMI, smoking, hemoglobin, albumin, CRP, IBD diagnosis, disease location at baseline, disease duration, and biological history), (2) fecal sample characteristics (covering the previously identified Bact2-associated stool features: microbial load, moisture content, and calprotectin concentration)[6,31] and microbiome data, (3) enterotypes, and (4) quantitative genera abundances. These sets of input variables were combined into 3 additional sets: (5) clinical data plus fecal sample characteristics, (6) set 5 plus enterotypes, and (7) set 6 plus quantitative genera abundances. Choice of biological therapy (a-TNF, VDZ, or UST) was always included as input.

Using the full set of 281 baseline profiles, we computed models to predict treatment outcome based on the 7 sets of input variables. Performances were compared by 5-fold cross-validation (training set n = 225, test set n = 56), with statistical significance tested by data permutation (100 iterations). Comparing first the performance of prediction models using logistic regression or neural network on the full variable set revealed that logistic regressions outperformed the more complex modelling with neural networks (logistic regression, receiver operating characteristic area under the curve [ROC-AUC] = 69.6%; neural network, ROC-AUC = 66.3%, both $P = .0099$) (Supplementary Table 17). Applying logistic regressions, combination 6, combining patient's clinical data, fecal characteristics (microbial load, moisture, and calprotectin), and Bact2-carrier status was identified as best performing (ROC-AUC = 73.9%), with a balanced sensitivity (67.5%) and specificity (67.6%) (Figure 3*D* and Supplementary Table 17). Adding quantitative genera abundances in the input set reduced the performance of the classifier (ROC-AUC = 69.6%) below the null model performance, likely due to overfitting after the addition of 58 taxa as predictors.

Next, the best performing model was applied to predict success of alternative therapies in case of nonresponse to the biological initially prescribed (152 interventions ending in nonremission, 9 excluded due to missing data for prediction) (Supplementary Table 18). By doing so, 27.63% of current nonresponders were identified as likely to achieve remission with a-TNF, 10.53% with VDZ, and 8.55% with any alternative therapy (Figure 3*E* and Supplementary Table 18).

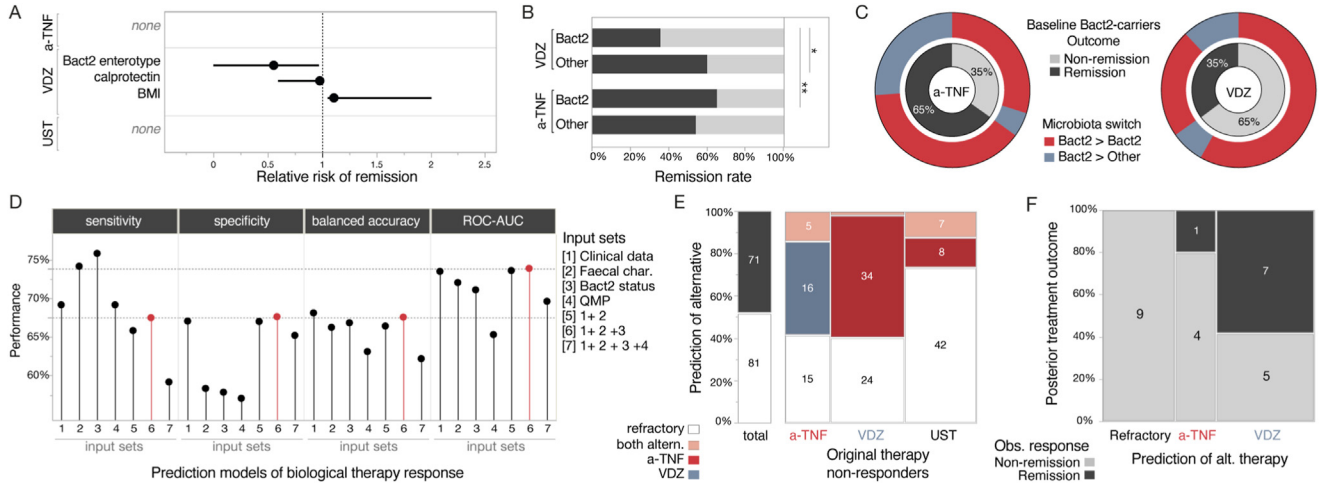

Figure 3. Biological therapy outcome evaluation by endoscopic remission and its prediction from patients' baseline characteristics (study cohort, np/ns = 253/281). (*A*) Modeling therapy outcome based on patients' clinical and gut microbiome characteristics at baseline (12 variables, n = 286, logistic regression). For significant predictors of therapy outcome, the associated relative risk of remission and 95% confidence interval are plotted as dot and whiskers. (*B*) Percentage of interventions with a-TNF and VDZ (n = 100, n = 118 interventions, respectively) resulting in endoscopic remission, stratifying patients with a baseline microbiota enterotyped as the dysbiotic Bact2-enterotype or other. *$P < .05$; **$P < .01$. (*C*) Pie chart representation of the observed rate of enterotype switch away from the Bact2-enterotype after biological therapy with a-TNF and VDZ (respectively n = 33 and n = 37 interventions in patients harboring baseline Bact2 microbiota), stratified by therapy outcome (remission/nonremission). (*D*) Performance metrics for logistic regression models built for the prediction of biological therapy outcome based on patients' baseline clinical status and fecal microbiota characteristics. Seven different sets of input variables were evaluated. The model performances (specificity, sensitivity, balanced accuracy, and ROC-AUC) evaluated by 5-fold cross-validation (training set n = 225, test set n = 56) are provided, with the best performance in red. QMP, quantitative microbiome profiling. (*E*) Prediction of alternative therapy outcome using the best performing model to reanalyze interventions having resulted in nonremission (n = 152 interventions). The model outputs the probability (*P*) of successful remission for each therapy, categorized as successful outcome ($P > 50\%$) with any alternative therapy (pink), a-TNF only (red), VDZ only (blue), or nonremission with any alternative therapy (refractory: white). (*F*) Validation of the alternative therapy outcome predictions in a small subset of 26 patients having had posterior interventions and their resulting endoscopic outcome evaluation. After a first failed intervention, the patients were predicted as refractory or responders for future a-TNF or VDZ therapy. The posterior interventions observation, resulting in remission (black), validate 65% of the predictions.

Although biological history was included in the classifier, predictions of a-TNF as an alternative therapy for VDZ- and UST-treated individuals are surprising and would require additional in vivo validation, because as in the present cohort, respectively, 72% and 92% of those patients were previously treated with a-TNF. Importantly, 81 of the 152 nonresponders (53.29%) were predicted to remain nonresponders with any alternative therapy, suggesting that a subset of patients would be refractory to any kind of biological intervention. For a small subset of 26 patients involved in the present study, information was available on posterior intervention and the resulting endoscopic response. This subset allowed some initial clinical validation of the model proposed. Predictions of response to the best alternative therapy had an overall 65.38% accuracy compared with the observed response to the second interventions, with 9 observed of 9 predicted nonremissions, and 8 observed of 17 predicted remissions (Figure 3*F*), in line with the results of the model validation presented above.

Discussion

The gut microbiota has been hypothesized to play a role in therapeutic responses in IBD. Recent longitudinal studies describing changes in the gut microbiome during biological treatment in IBD or correlating baseline composition to therapy outcome suggested a role for the gut microbial community as a modulator of treatment response and highlighted the potential of microbiome monitoring as a companion diagnostic approach.[13,18,19,21,25] Here, we extended the scope of these explorative analyses through the inclusion of 3 distinct classes of biological therapies in the study design and by focusing on endoscopic remission as an objective end point of the interventions assessed. Our analyses allowed us to identify disease location as a key microbiome covariate in baseline samples, with superior nonredundant explanatory power compared with the CD/UC diagnosis. A similar stratification of IBD patient groups has previously been suggested to result in more homogenous subgroups based on genetic analyses.[38–40]

Baseline prevalence of the dysbiotic Bact2 microbiota community type was higher in ileal compared with an exclusively colonic presentation. Fecal samples from patients with ileal disease were additionally characterised by increased abundances of *Fusobacterium* and *Bilophila*. *Fusobacterium* has previously been described to be associated with the inflammatory tone in ileal CD,[41,42] whereas Proteobacteria, such as *Bilophila*, have been shown to be

more prevalent in the small intestine of a canine model of IBD.[43] Moreover, a published quantitative analysis of microbiota variation in patients with CD and individuals with primary sclerosing cholangitis showed that the abundance of *Fusobacterium* correlated with fecal calprotectin concentrations.[6] Finally, the presence of *Fusobacterium* has also been linked to postoperative relapse in patients with CD after ileocecal resection[44] and reduced success rates of fecal microbiota transplantation in patients with UC.[45]

Regarding the association between the different biologicals and gut microbiota variation, our analyses demonstrated that only a-TNF treatment was accompanied by a shift in community composition over the course of the intervention. Moreover, within the limitations of our cohort, we only observed a-TNF treatment to result in a significant decrease in the prevalence of gut microbiota dysbiosis. In patients harboring a dysbiotic Bact2-enterotyped microbiota at baseline, a shift to other enterotypes was associated with successful remission with both a-TNF and VDZ. However, a baseline Bact2 carrier status was linked to a significantly lower probability of successful outcome of VDZ therapy, which was not the case for a-TNF.

Although our findings do not allow inferring causality, these observations may indicate that patients with baseline dysbiosis may benefit from a combined systemic anti-inflammatory and microbiota modulatory effect of a-TNF therapy. Our observations that a-TNF therapy would be a more effective option in patients harboring a dysbiotic gut microbiota was supported by the analysis of first-line interventions only; however, we cannot fully exclude bias in comparing biologicals in the present nonrandomized study design, with VDZ often prescribed to patients refractory to a-TNF. Moreover, it should be noted that our analyses do not allow us to establish a chain of events; that is, to determine whether restoration of eubiosis precedes, follows, or coincides with endoscopic remission. Additionally, a more prolonged period of fecal sample collection would be required to determine persistence of microbiota compositional changes and association to sustained remission; however, previous studies with a 1-year follow-up have shown that patient compliance decreases rapidly over time.[18]

Building on clinical data, nonsequencing-based fecal characterization probing dysbiosis-associated stool features[6,31] as well as detection of the potentially dysbiotic Bact2 enterotype, we were able to create a reproducible therapy-specific model predicting response to biological therapies. Our findings suggest that stool characterization combining quantification of microbial load, moisture content, and fecal calprotectin concentrations with enterotyping could be further integrated into a simple and straightforward tool for personalizing therapy and predicting therapeutic outcome. Intriguingly, based on the model, >50% of nonresponders were projected to fail to respond to any alternative treatment, thus identifying a subset of patients with high likelihood of being refractory to anti-inflammatory intervention. These results are in line with the conclusions of a recent meta-analysis of phase 3 randomized controlled trials with biologicals in IBD, showing that primary nonresponders to a-TNF treatment have a higher likelihood of not responding to other biological agents.[46]

Conclusion

Overall, our study has demonstrated a firm link between disease location and prevalence of microbiota dysbiosis, with implications for development of personalized therapeutic approaches as well as IBD research. In individuals hosting a dysbiotic community type, we observed remission was associated with microbiota shift to a eubiotic configuration. However, a-TNF treatment was shown to have a higher likelihood than VDZ of promoting remission in patients harboring a baseline dysbiotic gut microbiota. Finally, we demonstrated that a combination of clinical data, stool characteristics (microbial load, moisture content, and calprotectin concentration), and fecal enterotype (Bact2 vs other) allowed us to predict treatment response to biological therapies but classified more than half of patients with IBD as refractory to all anti-inflammatory therapies studied.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2023.11.304.

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Jeroen Raes, PhD (Conceptualization: Equal; Supervision: Lead; Writing – review & editing: Lead)

Sara Vieira-Silva, PhD (Methodology: Equal; Supervision: Lead; Writing – original draft: Equal; Writing – review & editing: Lead)

Severine Vermeire, MD, PhD (Conceptualization: Equal; Data curation: Equal; Supervision: Lead; Writing – review & editing: Lead)

Conflicts of interest
These authors disclose the following: Bram Verstockt reports financial support for research from Pfizer, lecture fees from AbbVie, Biogen, Chiesi, Dr Falk Pharma, Ferring, Galapagos, Janssen, MondayNight IBD, MSD, Pfizer, R-Biopharm, Takeda, and Truvion, and consultancy fees from Janssen, Guidepont, and Sandoz. Kathleen Machiels reports employment as a Pfizer medical advisor in inflammation and immunology since May 1, 2021. João Sabino has received speaker fees from Falk, AbbVie, and Takeda, and consultancy fees from Janssen and Fresenius Kabi. Marc Ferrante has received grants from AbbVie, Amgen, Biogen, Janssen, Pfizer, and Takeda, lecture fees from AbbVie, Amgen, Biogen, Boehringer-Ingelheim, Falk, Ferring, Janssen, Lamepro, MSD, Mylan, Pfizer, Sandoz, Takeda, and Truvion Healthcare, and consultancy fees from AbbVie, Boehringer-Ingelheim, Celltrion, Janssen, Lilly, Medtronic, MSD, Pfizer, Sandoz, Takeda, and Thermo Fisher. Jeroen Raes has received grants from Beneo, Cargill, Colruyt group, Danone, DSM, Johnson & Johnson, MRM/Prodigest, Nestlé Pfizer, and Takeda, and has received consulting or speaking fees, or both, from Aphea, Biofortis, DSM, Ferring, GSK, Janssen Pharmaceuticals, Metagenics, MSD, MRM/Prodigest, Nutricia, Takeda, and Tsumura. Séverine Vermeire has received grants from AbbVie, Johnson & Johnson, Pfizer, and Takeda, and has received consulting or speaking fees, or both, from AbbVie, Arena Pharmaceuticals, Avaxia, Boehringer-Ingelheim, Celgene, Dr Falk Pharma, Ferring, Galapagos, Genentech-Roche, Gilead, Hospira, Janssen, Mundipharma, MSD, Pfizer, Prodigest, Progenity, Prometheus, Robarts Clinical Trials, Second Genome, Shire, Takeda, Theravance, and Tillots Pharma AG. Séverine Vermeire, Sara Vieira-Silva, Jeroen Raes, Gwen Falony, and João Sabino are listed as inventors on patent W02019115755A1 "A new inflammation-associated, low cell count enterotype," in the name of Vlaams Instituut voor Biotechnologie (VIB) VZW, Katholieke Universiteit Leuven, Katholieke Universiteit Leuven R&D, and Vrije Universiteit Brussel, covering the features of the microbiome associated with inflammation described in reference 6. The remaining authors disclose no conflicts.

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Data Availability
Raw amplicon sequencing data that support the findings of this study have been deposited in European Nucleotide Archive (ENA) with accession codes PRJEB71738 with public access. Supplementary Material is available in the online version of the study.

Prior Presentation
Parts of these data were presented as a plenary oral presentation at the 15th Congress of the European Crohn's and Colitis Organisation, February 12–15, 2020, Vienna, Austria.